Species, Vol. 18, No. 58, January-March, 2017

RESEARCH

ISSN 2319–5746 EISSN 2319–5754

Species
An International Journal

Morphotaxometry and molecular heterogeneity of *Sturdynema multiembryonata* gen. et sp.n. (Spiruroidea : Gnathostomatinae) of fresh water garfish, *Xenentodon cancilla* from the Gangetic riverine ecosystem in northern India with a revised key to genera of Gnathostomatinae

Sushil Kumar Upadhyay☼

Department of Zoology, Keral Verma Faculty of Science, Swami Vivekanand Subharti University, Meerut- 250 005, Uttar Pradesh, India

☼Correspondence to: Dr. Sushil Kumar Upadhyay, Assistant Professor, Department of Zoology, Keral Verma Faculty of Science, Swami Vivekanand Subharti University, Meerut- 250 005, Uttar Pradesh, India, Email: upadhyay.k.sushil@gmail.com

Publication History
Received: 18 November 2016
Accepted: 26 December 2016
Published: January-March 2017

Citation
Sushil Kumar Upadhyay. Morphotaxometry and molecular heterogeneity of *Sturdynema multiembryonata* gen. et sp.n. (Spiruroidea : Gnathostomatinae) of fresh water garfish, *Xenentodon cancilla* from the Gangetic riverine ecosystem in northern India with a revised key to genera of Gnathostomatinae. *Species*, 2017, 18(58), 1-13

ABSTRACT

The two years consecutive investigations of fresh water garfish, *Xenentodon cancilla* (Teleostomi: Belonidae) were conducted for the helminthes infection of zoonotic significance off the Gangetic riverine ecosystem. The advanced 3rd stage larval forms and mature worms of *Sturdynema multiembryonata* gen. et sp.n. (Spiruroidea: Gnathostomatinae) were recovered found embedded in a cavity

within the liver tissues of fish. The autogenic infectivity being reported in life cycle typically within the aquatic hosts was remarkable. The morphotaxometric analysis and ultra topological investigations through scanning electron microscopy of proposed newer genera were validated by the critical appraisal of molecular heterogeneity on the basis of 18S rDNA analysis been presented in this investigation. The phylogenetic interrelationship of the newer worms worked out with the earlier existing genera of family Gnathostomatinae and revealed their significant differences with *Raphidascaris*, *Anisakis*, *Goezia, Porrocaecum* and *Terranova*. The worms, being reported here, with unique characteristics closer to Gnathostomatinae have never been reported from fish definitive hosts therefore, a revised key to genera of Gnathostomatinae has been mentioned in the current study. The distinguishing habitat, morphometric, ultra toplogical and phylogenetic features reported here, can therefore, be used to assign the present specimen to a new genera under family Gnathostomatinae.

Keywords: *Sturdynema multiembryonata* gen. et sp.n., *Xenentodon cancilla*, Morphotaxometry, Molecular heterogeneity, Ultra topology.

1. INTRODUCTION

Helminthes are a natural episode and common in most of the aquatic vertebrates. The invertebrates eaten by the fishes as prey within the habitat, may serve as vector or paratenic hosts or intermediate hosts for trophically transmitted helminthes parasites (nematodes), however, few roundworms can be transmitted directly from fish to fish. Nematodes are mostly niche specific in habit and can infect almost any part of the body, including the coelomic (body cavity) regions, visceral organs, swim bladder, deeper layers of the skin or fins and external muscle layers. The inhabitance of spiruroids, *Gnathostoma* have frequently been reported world over from variety of hosts (Miyazaki and Dunn, 1965; Yadav and Tandon, 1994; Camacho et al., 2002; Imai and Hasegawa, 2001; Bertoni-Ruiz et al., 2005; Youn, 2009; Almeyda-Artigas et al., 2010; Shrivastav et al., 2011; Tandon, 2011). The reports of fish as definitive hosts to harbor adult nematodes bearing gnathostomatoid characters have been scarce (Sieu et al., 2009). But few workers investigated fish as natural second intermediate hosts of *G. spinigerum* (Rojekittikhun et al., 2002). Recently helminthologists identified fish as a source to human infections in an indirect manner by discovery of infective advanced third-stage larvae of *G. nipponicum* in largemouth bass, *Micropterus salmoides*, *G. binucleatum* in Mexican fish, *viz. Petenia splendida, Cichlasoma managuense* and *Gobiomorus dormitory* (Kifune et al., 2004). The worms, being reported here, with unique characteristics closer to Gnathostomatoidea have never been reported from fish definitive hosts. The autogenic pattern of infection being investigated in life cycle classically within the aquatic hosts was remarkable. The phylogenetic interrelationship of the newer worms has been presented in this investigation on the basis of conserved gene sequence analysis to validate morphotaxometry worked out by the conventional method.

2. MATERIALS AND METHODS

Collection, examination and morphometry of worms

The investigations were conducted on fresh water garfish *Xenentodon cancilla* of Gangetic riverine ecosystem ($81^0$49’06.28”E (Lon), $25^0$24’53.24”N (Lat), 74m (Alt)). The hosts were harvested by angling netting methods and brought to laboratory for further studies (Upadhyay, 2012). The worms recovered from the liver (Figure 1) and muscles were further processed, fixed (Rautela and Malhotra, 1984) and mounted for morphotaxometric analysis (Upadhyay et al., 2009; 2015). Microphotographs were taken by image analyzer unit “MOTIC” and drawings were made with camera lucida (SIPCON SP-14). The ultra topology of collected worms conducted and observed under variable pressure Scanning Electron Microscope (LEO 435 VP) and microphotographs were captured at different magnification after Upadhyay (2012). All the morphometric measurements are given in micrometers (μm) as ranges followed by mean ± standard error (SE) in parentheses.

Molecular and phylogenetic analysis

The morphotaxometric ambiguity in identification of helminth taxa were resolved by the molecular phylogenetic analysis. The genomic DNA was extracted through techniques of molecular taxonomy (Sambrook et al., 1989; 2001) from individual worms. The

validation of taxa was based on the analysis of highly conserved gene sequences of individual worms. The highly conserved 18S rRNA gene was amplified by Polymerase Chain Reaction (PCR) technique (Cutillas et al., 2004). The primers used for the amplification of 18S rRNA gene were: Nem18SF, Nem18SR (Floyd et al., 2005). Amplification of gene was performed as per standardized protocol (Upadhyay, 2012). A total of 50ng concentration of amplified PCR product was used for sequencing (Hu and Gasser, 2006). The purification of products was done by Centri-Sep spin columns (Collegon et al., 2009) and sequenced by ABI 3730 autoseqencer (Applied Biosystems). The obtained sequences were edited in BioEdit (Hall, 1999) and compared by using computational technique, BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1990). The neighbor joining phylogenetic dendrogram (Saitou and Nei, 1987) and Kimura's two parameter model evolutionary distances (Kimura, 1980) were computed by MEGA version 4.0 (Tamura et al., 2007) as well as Clustal W computer program (Thompson et al., 1997).

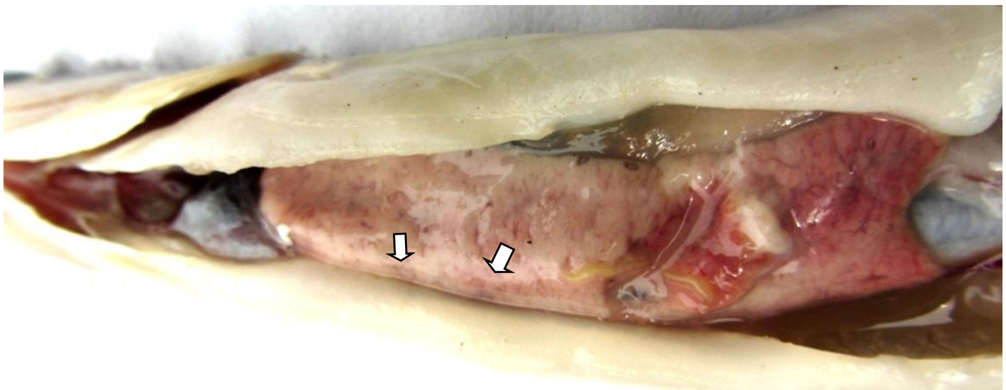

Figure 1

Mature *Sturdynema multiembryonata* gen. et sp.n. in liver of *Xenentodon cancilla*.

3. RESULTS

Description

Body enclosed in a cuticular envelope with a marked cephalic bulb, without rows of spines, bearing 3 lips (Figures 2A,B,C,D; 3D; 4A,B; 5A). The cephalic bulb distinctly demarcated from rest of the body by muscular collar (Figure 2A,B). The inner margin of each lip was trilobed (Figure 2A,B,C). Each lip has 3 pairs of cephalic papillae (Figure 2B,F). The trilobed lip structure on head was unique in this newly recovered worm. Each lip had 3 basal inward extensions that projected into the buccal cavity, while proximally they represented single lobe of lip, 3 of which merged to form a larger lip on which cephalic papillae were located. Each projections extending from the lips had a broader basal structure proximally, that appeared as cylindrical laterally and a knob-like distal part that was hanging freely over the buccal cavity (Figure 2A,B,C,F). Sharp elongated spines of varied sizes present in distinct horizontal rows on the cuticular envelope, covering the body surface from the base of the cephalic bulb extending upto tip of the tail (Figures 2,3,4,5). Caudal alae prominent (Figures 3E,G,H; 4C,D; 5C). Excretory pore opens latero-ventrally (Figure 5A), located between 21st and 23rd rows of spines in the forebody. Cervical papillae 1 pair located between 17th and 18th rows of spines from anterior extremity. Cervical sacs 2 pairs extending upto 24-42nd rows of spines (Figures 4B,5A). Nerve ring located between 14th and 15th rows of spines in the forebody. The hind extremity of the oesophagus was followed a smaller bulbar ventriculus (Figure 5B). The intricate cuticularized valvular configuration was distinct at the junction of the oesophagus and intestine. The body reaches greatest width at about 2/3rd from anterior end of body.

The structure of spines just behind cephalic bulb and at the region of oesophageo-intestinal junction did not have any specialized anatomical feature, and showed irregularly alternate placement of longer and smaller-sized spines in each row on the body (Figures 2,3). However, the ornamentation on body, beginning from 63-64 rows behind cephalic bulb, that extended upto 104th row from the tail extremity, showed irregularly spaced clusters of 2-4 spines clubbed together on a muscular base (Figure 3C) and at times there occurred single spines too in between such clusters (Figure 2G). Such spine clusters were interspaced by regular elongated spines in each row, with a few smaller spines in between. A pair of knob-like protuberances with cluster of unequal spines occurred in mid-body rows of spines of adult male worms (Figure 3A,B). The total numbers of horizontal rows of spines were, 201 in

males and 174 in females, on whole body of worms. The distribution of smaller-sized spines nearer to pre-anal area comprised utmost two spines with a joint base with irregularly-spaced single spines, in between, in the same row (Figures 2H, 3A). Various measurements of spines and different body organs of mature worms of the proposed new genus are summarized in Table 1.

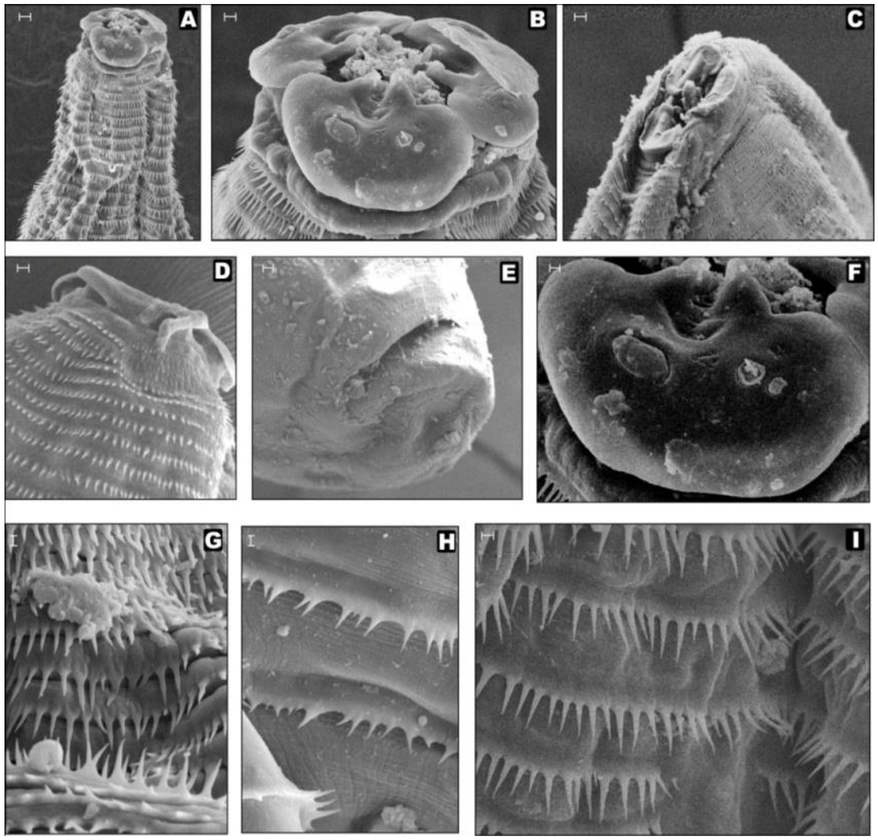

Figure 2

Sturdynema multiembryonata gen. et sp.n. A- Anterior end of mature worm; B,C- Anterior end with cephalic bulb, lips and collar; D- Anterior end of female worm; E- Posteror end of female worm with tail process; F- Trilobed lip with cephalic papillae; G- Spines at the oesophageal region; H- Spines at posterior end of body; I- Spines at middle region of body.

Table 1 Morphometric measurements of *Sturdynema multiembryonata* gen. et sp.n.

Character		Males	Females
Body	L	5916-18281 (11281±619)	14823-47345 (24161±1838)
	W	268-1377 (814±31)	882-3978 (1884±79)
Head	L	27-85 (58±4)	36-106 (71±8)
	W	102- 266 (195±9)	198-360 (283±10)
Lips	L	68-85 (77±9)	67-108 (83±14)

	W	102-164 (128±11)	117-162 (135±39)
Buccal cavity	L	29-81 (53±7)	81-225 (147±12)
	W	89-153 (126±10)	144-306 (225±13)
Glandular oesophagus	L	228-768 (425±36)	475-1188 (859±41)
	W	102-307 (210±11)	198-864 (456±30)
Muscular oesophagus	L	285-873 (583±46)	810-1923 (1168±57)
	W	186-843 (413±29)	450-1206 (778±32)
Oesophagus	L	748-1961 (1156±71)	1638-2448 (2059±79)
	W	162-919 (377±31)	198-1206 (639±43)
Oesophagus/body length%		4.976-15.418 (10.028±1.329)%	6.196-14.208 (9.705±0.613)%
Intestine	W	81-387 (215±22)	153-589 (350±21)
Distance of vulva-	a. from anterior end	-	4977-13644 (9290±733)
	b. from posterior end	-	8028-20790 (12589±824)
Eggs	L	-	35-180 (107±10)
	W	-	31-171 (89±11)
Left spicule	L	332-1006 (641±28)	-
	W	9-64 (33±5)	-
Right spicule:	L	322-765 (544±27)	-
	W	7-50 (26±4)	-
Ratio of right spicule to body length%		2.713-11.494 (6.393±0.485)%	-
Ratio of left spicule to body length%		2.631-8.064 (5.239±0.326)%	-
Distance of anus from tail tip		85-225 (147±11)	351-738 (488±18)
Tail process	L	27-189 (75±8)	49-126 (81±8)
	W	16-34 (25±5)	18-72 (42±8)
Pre-anal Papillae	No.	11-12	2
Ad-anal papillae	No.	1	-
Post-anal papillae	No.	6-7	-

Spines at cephalic rim	L	4-10 (7±1)	6.5-9.5 (8±1)
	W	2-3 (2.7±0.6)	3-5 (4±0.7)
Spines at tail tip	L	6.5-9.5 (7.9±1.4)	7-14 (10±1)
Space between rows of spines		18-104 (73±8)	18-108 (65±3)

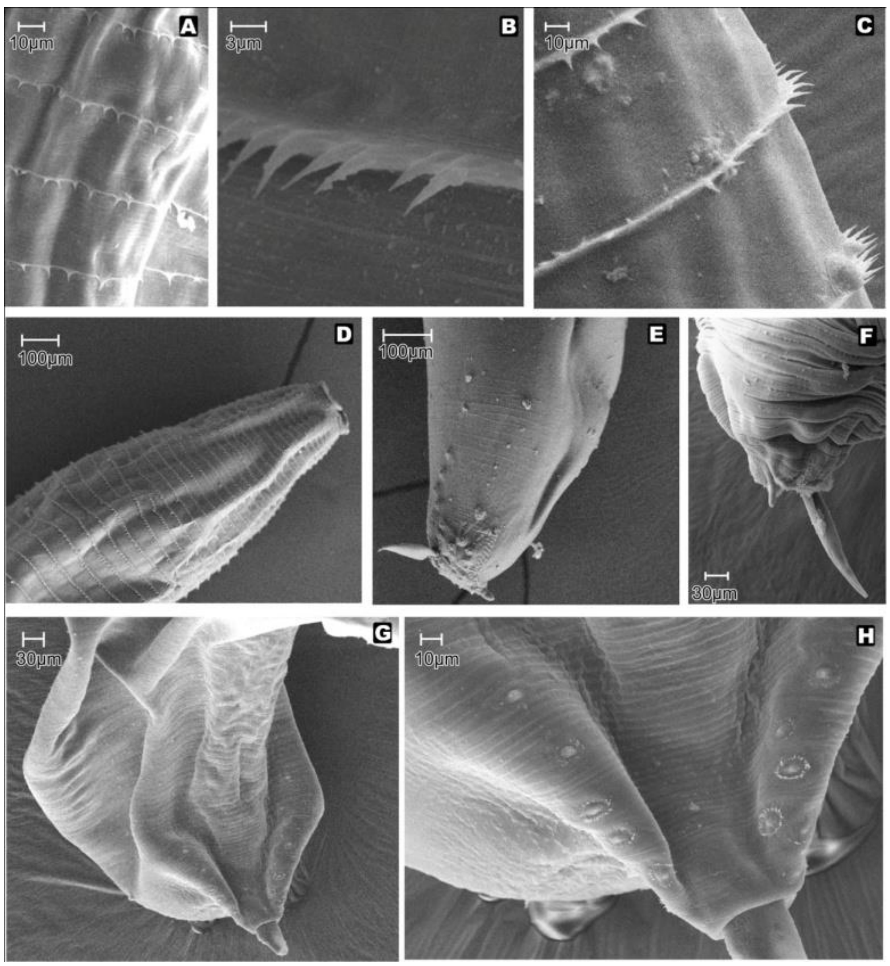

Figure 3

Sturdynema multiembryonata gen. et sp.n. A,B,C- Patterns of spines distribution in mature worms; D- Anterior end of male; E- Unpaired adanal papillae in male; F- Spicules and tail process in male; G,H- Distribution of pre-anal caudal papillae in male.

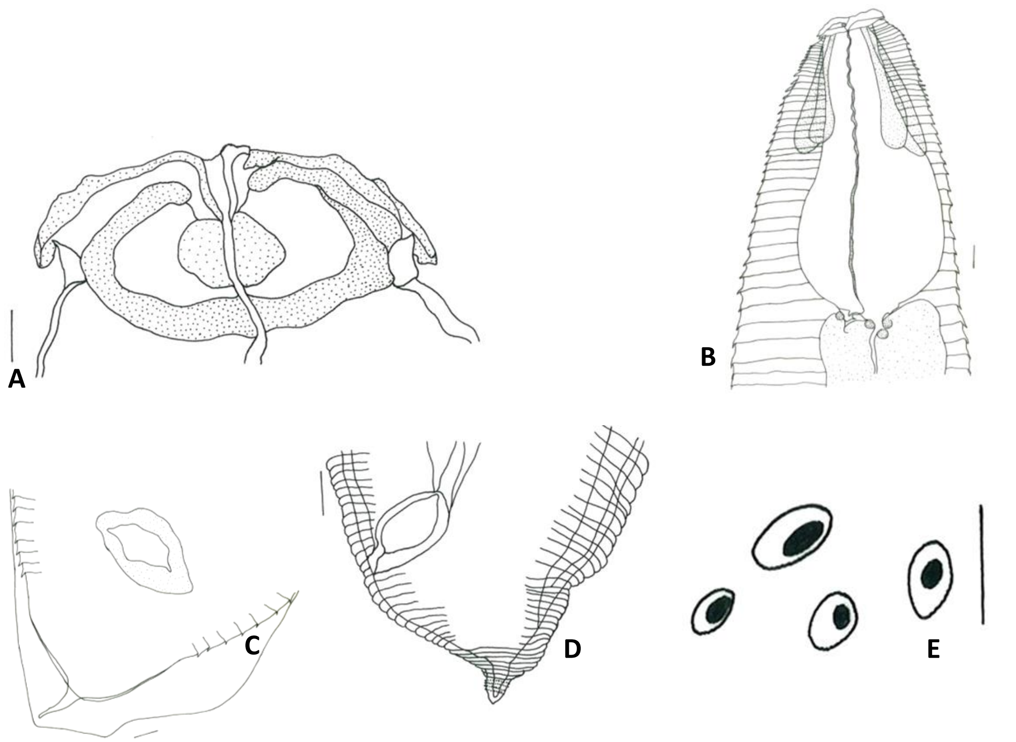

Figure 4

Sturdynema multiembryonata gen. et sp.n. (Scale bar= 0.10mm.). A- Cephalic bulb of mature worm; B- Anterior end of female with two pairs of cervical sac; C,D- Posterior end of female with tail process; E- Unipolar Eggs.

Male

The worms were comparatively smaller in size (Table 1) with unarmed collar like structure at the base of head (Figures 2A,B; 3D; 5A). Two long, sub-equal spicules (Figures 3F,5C) were present in adult worms. The caudal papillae (Figures 3E,5C) comprised total 6-7 pairs post-anal and 11-12 pairs pre-anal. Out of which one pair medial, while the terminal 4 pairs ventro-lateral ones were typically pedunculated with a rounded distal extremity, a distinctly marked muscular base supported at the base by 1 pair of elongated muscular flaps, arranged longitudinal to the body plane (Figures 3E,5C), and simultaneously encircled by irregularly alternate longer, sharp spines with a broader lower half, and simple smaller spines. The other 6-7 pairs dorso-lateral ones were sessile, encircled by smaller indistinct spines, that were not easily made out (Figure 3G,H). An unpaired elongated digitiform ad-anal papilla was present near anus (Figures 3E,5C), with the tail terminating into a short terminal process (Figures 3,5).

Female

Females worms were comparatively larger (Table 1). Vulva was pre-equatorial in position. Two pairs of pre-anal typical caudal papillae were observed on tail, one of these was pedunculate, ungulate, but the other sessile, and a short terminal process was present at the tail end (Figure 4). The eggs were unipolar and embryonated identified in the uterus of mature worms (Figure 4E).

Type host: *Xenentodon cancilla* (Teleostomi: Belonidae)

Site of infection: Liver, muscles

Type locality: River Ganges, Allahabad, Uttar Pradesh, India.

Type material: **Holotype,** ♂ ZSI/IN596; **Paratype,** ♀ ZSI/IN597 deposited with nematode collections, Zoological Survey of India, Dehradun, Uttarakhand, India.

Etymology: The new genus and species named after its sturdy appearance and multiembryonated stage in life cycle.

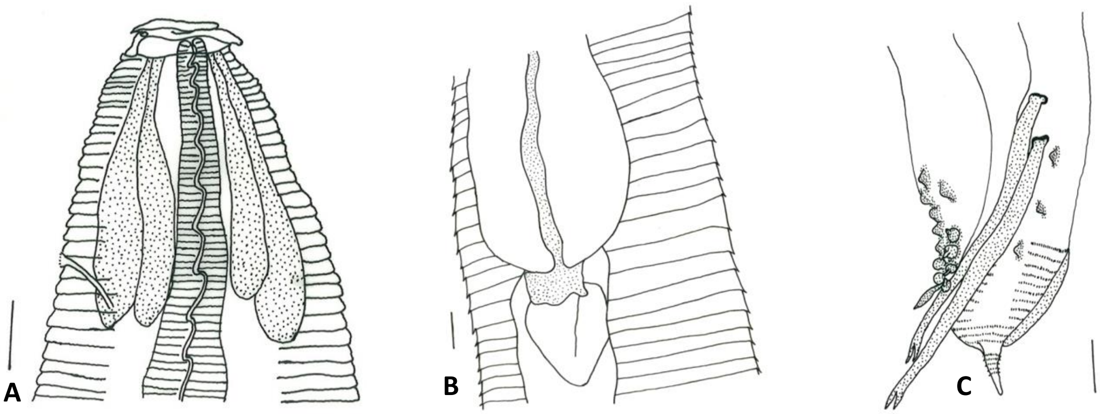

Figure 5

Sturdynema multiembryonata gen. et sp.n. (Scale bar= 0.10mm.). A- Anterior end of male with two pairs of cervical sac and excretory pore; B- Oesophageo-intestinal Junction; C - Posterior end of male with caudal papillae, spicules and tail process.

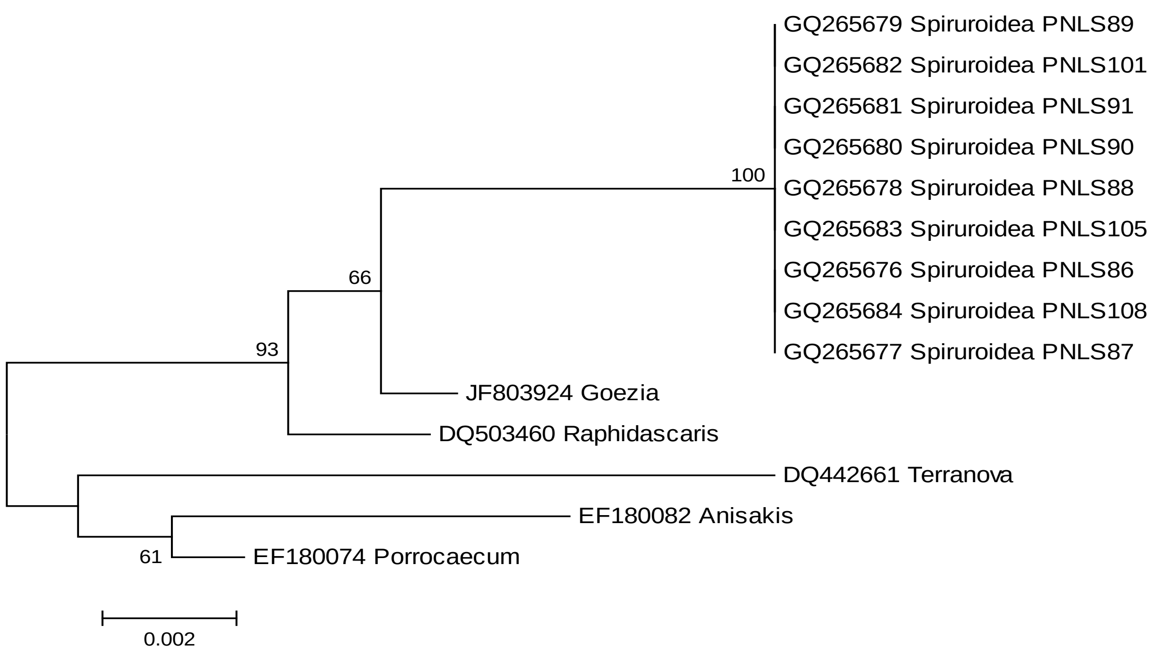

Figure 6

Neighbour joining tree based on nucleiotide 18S rRNA gene sequences of *Sturdynema multiembryonata* gen. et sp.n. (Spiruroidea) with reference species. Scale bar represents an interval of the Kimura two-parameter (K2P) model with bootstrap values, as based on 1000 replicates.

Molecular analysis, 18S rRNA gene

The read length of all 18S rDNA sequences were 863 bp, but no insertions and deletions were detected. The average K2P distance of individuals within *S. multiembryonata* gen et sp.n. based on 18S rRNA gene sequence was 0.0, as compared with an average distance of 0.007 with *Goezia*, 0.009 with *Raphidascaris*, 0.020 with *Anisakis*, 0.015 with *Porrocaecum* and 0.022 with *Terranova* respectively

(Figure 6). It may be noteworthy that there were 838 (97.1%) conserved domains and 14 parsimony informative sites within the 863 bp long 18S region of *S. multiembryonata* gen et sp.n. The overall GC content of newer worm was found to be 49%. Since 18S rDNA is a non-protein coding gene, but due to higher hydrogen bonding with complementary base pairs it play a noticeable significance in inferring the phylogenetics of this organism. A neighbor-joining tree was generated using the Kimura 2 Parameter to calculate pairwise distances. The nine specimens GQ265676-684 form a separate clade (Figure 6) which does not show any significant similarity to any other organism listed in the GenBank.

4. DISCUSSION

The characters of Gnathostomatinae, showed closest affinity to the worms of new genus, particularly presence of cephalic bulb, and the size and varied structure of spines, partially or all over the body (Miyazaki, 1960). Specimens in this study were closer in morphology of taxonomically important features to those described in *Gnathostoma* Owen, 1836 by Yamaguti (1962), Bertoni-Ruiz et al. (2011), Camacho et al. (2002). Immense taxonomic significance is attached to the number and shape of hooklets (Sohn and Lee, 1998), size and general body shape of worms, number of rows and arrangement of cephalic spines, size of caudal pedunculate papillae and Y-shaped spineless area at posterior extremity, in determination of species of *Gnathostoma*. Of the native species, it could be differentiated from *G. doloresi* in possessing single polar plug than on both ends; from *G. hispidum* in absence of wart-like cap on the single polar plug on eggs; and from *G. spinigerum* in the whole body being covered with spines of different sizes while only anterior two-thirds of the body of the former species were covered with spines. The cephalic spines in *G. spinigerum* were like claws of the cat, while cephalic region lacked spines in the specimens of the new genus, and those present on rest of the body were predominantly simple, elongated backwardly directed and essentially single-spined. The striking contrast of shape of spines, lacking palmate, bi- or tri-furcate structure of spines on body, 3 lips, each with 3 pairs of cephalic papillae, number of caudal papillae that were surrounded by specialized musculature and spination patterns at their base, pre-equatorial vulva and absence of Y-shaped spineless area at posterior extremity differentiated the specimens collected in the current study from all the established species of Genus *Gnathostoma*. Bertoni-Ruiz et al. (2011) held a total of 13 species as valid under genus *Gnathostoma* that is closest to the present worms. These include, *G. americanum* Travassos (1925), *G. binucleatum* Almeyda-Artigas (1991), *G. doloresi*, *G. hispidum, G. lamothei* Bertoni-Ruiz et al. (2011), *G. malaysiae* Miyazaki and Dunn (1965), *G. miyazakii* Anderson (1964), *G. nipponicum* Yamaguti (1941), *G. procyonis* Chandler (1942), *G. socialis* Leidy (1858), *G. spinigerum, G. turgidum* Stossich (1902), *G. vietnamicum* Le-Van (1965). Simultaneously Bertoni-Ruiz et al. (2011) upheld opinion of Miyazaki (1990) devalidating *G. brasiliensis* Ruiz, 1952 and *G. didelphis* Chandler, 1932. However, zoonotic potential was assigned to only 4 of these, *viz. G. spinigerum*, *G. hispidum*, *G. nipponicum* and *G. doloresi* (Sohn and Lee, 1996; Youn, 2009). But as regards the larger size of worms, specimens assigned to the new genus were closer only to two species, *viz. G. miyazakii* and *G. turgidum* in larger size of worms, particularly females.

A critical appraisal of results of molecular analysis of the newer roundworms revealed striking features of dissimilarity from various genera that apparently exhibited substantive differences morphologically as well. However, these worms were closer to *Raphidascaris* Railliet and Henry (1915) in possessing lips without dentigerous ridges and gubernaculum in male worms; a small ventriculus after oesophagus, pre-equatorial vulva and oviparous females, and parasitic in teleosts but the differences were obvious in well developed cuticular expansions in the latter, particularly on ventral lips and absence of interlabia; absence of posterior appendix from ventriculus, that followed oesophagus; absence of intestinal caecum; sub-equal (Vs equal), non-alate (Vs alate) spicules in male worms; a terminal tail process at the extremity of tail in female worms (Vs an attenuated tail). The newer worms also resembled with *Goezia* Zeder (1800) in possessing a series of rings on body cuticle, provided posteriorly with backwardly directed spines; lips flattened and expanded outwards, separated from body by a constriction; oesophagus slightly constricted in middle and swollen posteriorly into a bulb; sub-equal spicules, tail prolonged into an appendage in male worms, a number of preanal and a few postanal papillae; the blunt process on tail in females armed with spinelets, vulva pre-equatorial and oviparous females. But the differences of these worms from *Goezia* in absence of ventricular appendages in oesophagus and absence of intestinal caecum were noticeable. The worms of the new genus resembled *Porrocaecum* Railliet and Henry (1912) in possessing a ventriculus without posterior appendix, absence of intestinal caecum and gubernaculum and oviparous female worms, but differed from it in absence of dentigerous ridges on lips and interlabia, sub-equal spicules (Vs equal spicules) and vulva pre-equatorial (Vs near middle of body). The newer worms had resembling features similar to *Anisakis* Dujardin (1824) in absence of interlabia; ventricular appendix or intestinal caecum; pre-equatorial vulva and oviparous females, and differentiating characteristics to genus *Anisakis* in absence of dentigerous ridges on lips; possessing lesser number of postanal papillae; sub-equal spicules without spirally twisted (Vs unequal or

equal spicules that may be spirally twisted) and tail with a conical point (Vs a short tail process at the posterior extremity). The worms of the new genus also resembled *Terranova* Leiper and Atkinson (1914) in presence of ventriculus and pre-equatorial vulva, but the differentiating features of these worms from *Terranova* were absence of dentigerous ridges and absence of interlabia; absence of ventricular appendix and intestinal caecum, and excretory pore located between 21^{st}-23^{rd} rows of spines from anterior extremity of the worms (Vs between 2 sub-ventral lips).

The worms of the new genus resembled *Toxocara* Stiles (1905) in possessing lips with 3 lobes, oesophagus with distinct muscular bulb posteriorly, terminal digitiform appendage and caudal alae on tail of male, spicules sub-equal, pre-equatorial vulva in oviparous female worms, and oviparous female worms as well as absence of interlabia and gubernaculum. The worms of the new genus differed from *Toxocara* in absence of cervical alae and dentigerous ridges on lips, non-alate spicules, eggs without pitted surface, and parasitic in freshwater fish under natural conditions (Vs carnivores and elephants). The striking difference in presence of 4^{th} moulting stage in *Toxocara* was remarkable, as the 3^{rd} moulting stage of newer worms transformed directly into adult worms.

The comparison of the newer worms from specimens of other closer species of genus *Gnathostoma* also revealed that the nerve ring was located comparatively at a farther distance in number of rows of spines (14-15 Vs 12-13) than in *G. miyazakii;* cervical papillae were, however, closer (17-18 rows of spines Vs 18-22) than *G. miyazakii* and excretory pore was also closer (21-23 rows of spines Vs 27-29) than in *G. miyazakii*. The nerve ring of the worms of new genus was located farther (14-15 rows of spines Vs 9-10) than its location in *G. turgidum*, while cervical papillae were located closer (17-18 rows of spines Vs 24-26) than in *G. turgidum*. The nerve ring of the worms of new genus was located closer (14-15 rows of spines Vs 21-22) than its location in *G. lamothei*, while cervical papillae were located farther (17-18 rows of spines Vs 7-11) than in *G. lamothei*. The excretory pore was closer (21-23 rows of spines Vs 25-28) than in *G. doloresi*. Simultaneously, cervical papillae were farther (17-18 rows of spines Vs 10-13) than *G. hispidum* and excretory pore was also farther (21-23 rows of spines Vs 19-20) than in *G. hispidum*. The excretory pore of the new genus was closer (21-23 rows of spines Vs 22-28) than in *G. spinigerum*, as well as in *G. binucleatum* (21-23 rows of spines Vs 23-24 (Ash, 1962), 24-28 (Koga, 1996) and about 30 (Lamothe-Argumedo et al., 1989). The unequal spicules in *G. americanum, G. binucleatum, G. doloresi, G. hispidum, G. lamothei, G. malaysiae, G. miyazakii, G. procyonis, G. socialis, G. spinigerum* and *G. turgidum* were also differentiable from the worms of the proposed new genus, while subequal spicules were recorded in the latter. The lack of spination in the 2^{nd} half of body of worms of *G. americanum*, *G. lamothei, G. nipponicum* (tail in female aspinous but in male spinous (Lee et al., 1988), no 'Y'- shaped aspinous area around cloaca in *G. spinigerum* Lee et al. (1988), *G. turgidum*, *G. vietnamicum,* was also a pertinent differentiating feature of the newly proposed genus. The life cycle of the proposed newer genus was further comparable with the species of *Gnathostoma* in that the presence of multiple binary fission has not been reported from the latter, but on this account the former resembled genus *Trichinella,* in which multiple binary fission has been reported. However, the differentiating features in the latter were polar plugs at both ends of eggs (Vs single polar plug in the new genus), single spicule in male worms (Vs 2 subequal ones in the new genus), and viviparous females (Vs oviparous females in the new genus). The most striking feature in which the worms of the new genus came closer to *G. miyazakii* and *G. lamothei* were the roundish bosses in the posterior part of body. But these structures were present only in males of *G. miyazakii*, while in the latter species these structures were present in both sexes of worms (Bertoni-Ruiz et al., 2005), as was seen in the new genus. Though roundish bosses were present in *G. miyazakii* but the spines were lacking in the posterior region, unlike the present worms. The spines were found in the caudal region of *G. lamothei*. The terminal digitiform appendage in the worms collected from fish of river Ganges resembled to a similar entity in *G. miyazakii* but the caudal region was profusely spinated, unlike the latter species. Simultaneously, the posterior end of the present specimens was not widened, like it was recorded in *G. vietnamicum.* Koga et al. (2003) emphasized that the egg shell of the Mexican species of *Gnathostoma* were not pitted, but those of *G. spinigerum* and other gnathostome species had many pits on the surface (Koga, 1996). On the contrary, worms of the proposed new genus did not have pits on the surface of egg shell, and thus differed from the type species, *G. spinigerum* as well.

The differences in morphology of the worms were amply supported by 18S rDNA sequence differentiation. Therefore, on the basis of the aforementioned significant points of morphological differences, distinctive 18S rDNA sequences being presented simultaneously. The variance in GC content among *S. multiembryonata* gen et sp.n. was higher in the case of the third base (42.2%) as compared with the first (21%) and second base (39%). Nucleotide sequence data of *S. multiembryonata* gen et sp.n. are available in GenBank database as spiruroidea under the accession numbers, GQ265676, GQ265677, GQ265678, GQ265679, GQ265680, GQ265681, GQ265682, GQ265683 and GQ265684. Therefore, the barcode structure, including detailed analysis of phylogeny of worms, based on information of 18S rRNA gene, was worked out to propose the newly collected worms, for inclusion under the

newly proposed genus, *Sturdynema* as a new species, *S. multiembryonata* gen. et sp.n. The revised key to genera of Gnathosomatinae was thus proposed as given below:-

Key to genera of Gnathostomatinae

a. Cephalic bulb divided into 2 or 4 lobes and furnished with transverse cuticular ridges with sharp, posteriorly projecting edges but without hooks. Parasites of reptiles……………………………………………*Tanqua* Blanchard, 1904

Cephalic bulb undivided externally and armed with transverse rows of recurved hooks………………………………**b**

b. Body unarmed. Parasites of fishes…………………………………………………………………………*Echinocephalus* Molin,1858

Body partially or wholly unarmed posteriorly directed spines. Parasites of fishes or mammals……………………**c**

c. Cephalic bulb with 1 pair lips, vulva post-equatorial, caudal papillae less than 15 in number. Parasites of mammals……………… ……………………………………………………………………………………………*Gnathostoma* Owen,1836

Cephalic bulb with 3 lips, vulva pre-equatorial, caudal papillae upto 38-45. Parasites of fishes and mammals ……*Sturdynema* gen. et sp.n.

5. CONCLUSION

The recovered roundworms were of great zoonotic significance off the Gangetic riverine ecosystem found embedded in a crater within the liver of fresh water garfish, *X. cancilla*. The conventional morphotaxometric and ultra topological findings were validated by the decisive judgment of molecular phylogenetics on the basis of highly consereved 18S rDNA analysis. The phylogenetic interrelationship of the newer worms worked out with the earlier existing genera of family Gnathostomatinae and revealed their significant differences. The worms, taken in consideration during investigation have unique characteristics closer to Gnathostomatinae that have never been reported from fish definitive hosts. Therefore, a revised key to genera of Gnathostomatinae has been mentioned in the current study. Thus on the basis of distinctive habitat, morphometric, ultra toplogical and phylogenetic features the collected worms were anticipated for inclusion under the newly proposed genus, *Sturdynema* as a new species, *S. multiembryonata* gen. et sp.n. under the family Gnathostomatinae.

DISCLOSURE STATEMENT

There was no special financial support for this research work from the funding agency.

ACKNOWLEDGEMENT

SKU is thankful to Prof. S. K. Malhotra, Head, Department of Zoology, University of Allahabad (A Central University), Allahabad, Uttar Pradesh, India for his able guidance and laboratory facility during the period of investigation. Author would also like to express his sincere thanks to Sophisticated Analytical Instrumentation Facility (SAIF-DST), Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi, India for the Scanning Electron Microscopy (SEM) of newer worms.

REFERENCES

1. Almeyda-Artigas RJ. Hallazgo de *Gnathostoma binucleatum* n.sp. (Nematoda: Spirurida) en felines silvestres y el papel de peces dulceacuicilas y oligohalinos como vectores de la gnathostomiasis humana en la cuenca baja del Rio papaloapan, Oaxaca-Veracruz, Mexico. *Annal inst Cien Mar Limnol Univ Nac Auto Mexico*, 1991, 18, 137-155
2. Almeyda-Artigas R J, Mosqueda-Cabera MA, Sanchez-Nunez E. Precocity of *Gnathostoma turgidum* in naturally infected four-eyed opossum *Philander opossum pallidus* from Temascal, Oaxaca, Mexico. *Parasitol Res*, 2010, 106, 439-443
3. Altschul SF, Gish W, Miller W, Myres EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*, 1990, 215, 403-410
4. Anderson RC. *Gnathostoma miyazakii* n.sp. from the Otter (*Lutra canadensis*) with comments on *G. sociale* (Leidy, 1858) of Mink (*Mustela vison*). *Canad J Zool*, 1964, 42, 249-254
5. Ash LR. Migration and development of *Gnathostoma procyonis* Chandler, 1942, in mammalian hosts. *J Parasitol*, 1962, 48, 306-313
6. Bertoni-Ruiz F, Argumedo MRLY, Garcia-Prieto L, Osorio-Sarabia D, Leon-Regagnon V. Systematica del genero *Gnathostoma* (Nematoda: Gnathostomatidae) en America. *Rev Mex Biodiv,* 2011, 82, 453-464
7. Bertoni-Ruiz F, Garcia-Prieto L, Osorio-Sarabia D, Leon-Reganon V. A new species of *Gnathostoma* (Nematoda:

Gnathostomatidae) in *Procyon lotor hernandezi* from Mexico. *J Parasitol*, 2005, 91, 1143-1149
8. Blanchard E. *Tanqua* n.g., replacement *Ctenocephalus* Von Linstow. *Arch Parasitol*, 1904, 478p
9. Camacho DSP, Willms K, Ramos MZ, Otero MCC, Nawa Y, Akahane H. Morphology of *Gnathostoma* sp. isolated from natural hosts in Sinaloa, Mexico. *Parasitol Res*, 2002, 88, 639-645
10. Chandler AC. Notes on the helminth parasites of the opossum (*Didelphis virginiana*) in Southeast Texas, with descriptions of 4 new species. *Proc US Nat Mus*, 1932, 81, 1-15
11. Chandler AC. The helminthes of raccoons in east Texas. *J Parasitol*, 1942, 28, 255-268
12. Collegon R, Rozas M, Ariza C, Ubeda JM, Guevara DC, Cutillas C. Cytochrome oxidase subunit-I and mitochondrial 16S rDNA sequences of *Trichuris skrjabini* (Tricocephalida: Trichuridae). *Parasitol Res*, 2009, 104, 715-716
13. Cutillas C, Oliveros R, Rozas M, Guevara DC. Determination of *Trichuris skrjabini* by sequential of the ITS1-5.8S-ITS2 segment of the ribosomal DNA. Comparative molecular study of different species of Trichurids. *J Parasitol*, 2004, 90, 648-652
14. Dujardin F. *Anisakis* sp. The Nematodes of Vertebrates. *In Systema Helminthum Vol III*. (Ed. By S Yamaguti), 1824, 1261p. Interscience Publications Inc NY,
15. Floyd RM, Rogers AD, Lambshead PJD, Smith CR. Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Mol Ecol Notes*, 2005, 5, 611-612
16. Hall TA. Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl Acids Symp Ser*, 1999, 41, 95-98
17. Hu M, Gasser RB. Mitochondrial genomes of parasitic nematodes- progress and perspective. *Trend Parasitol*, 2006, 22, 78-84
18. Imai J, Hasegawa H. Molting of *Gnatostoma doloresi* (Nematoda: Gnathostomatoidea) in the definitive host. *J Parasitol*, 2001, 87, 14-18
19. Kifune T, Lamothe-Argumedo R, Gracia-Prieto L, Oceguera-Figueroa A, Leon-Regagnon V. *Gnathostoma binucleatum* (Spirurida: Gnathostomatidae) en peces dulceacuicolas de Tabasco, Mexico. *Rev Biol Trop*, 2004, 52, 34-44.
20. Kimura, M. A simple method for estimating evolutionary rate of base substitutions through comparative studies on Nucleotide sequences. *J Mol Evol*, 1980, 16, 111-120
21. Koga M. Comparative surface ultrastructure of adults and eggs of *Gnathostoma* obtained in Japan. *Southeast Asian J Trop Med Pub Hlth*, 1996, 27, 71-74
22. Koga M, Akahane H, Lamothe-Argunedo R, Osorio-Sarabia D, Gracia-Prieto L, Martinez-Cruz JM, Camacho, SPD. Surface ultrastructure of larvae and adults of *Gnathostoma binucleatum* obtained in Mexico. *Bull Cent Res Inst Fukuoka Univ*, 2003, 1, 1-8
23. Lamothe-Argumedo R, Medina-Vences RI, Lopez-Imenez S, Gracia-Prieto I. Hallazgo de la forma infective de *Gnathostoma* sp., en peces de Temazcal, Oaxaca, Mexico. *Annal Inst Biol Univ Nac Auto Mexico Ser Zool*, 1989, 60, 311-320
24. Lee SH, Hong ST, Chai JY. Description of a *Gnathostoma spinigerum* recovered from a Thai woman with meningoencephalitis. *Korean J Parasitol*, 1988, 26, 33-38
25. Leidy J. *Cheiracanthus socialis* in the stomach of mink (*Mustela vison*). *Acad Nat Sci Philadelphia*, 1858, 2, 4p
26. Leiper RT, Atkinson EL. Helminthes of the British Antarctic Expedition, 1910-1913. *Proc Zool Soc London*, 1914, 222-226
27. Le-Van H. Etude d'un noveaux gnathostome *Gnathostoma vietnamicum* n. sp. chez *Lutra elioti* du Viet-Nam. *Bull Soc Pathol Exot*, 1965, 58, 228-235
28. Miyajaki I, Dunn FL. *Gnathostoma malaysiae* sp.n. from rats on Tioman Island, Malaysia (Nematoda: Gnathostomatidae). *J Parasitol*, 1965, 51, 382-384
29. Miyazaki I. On the genus *Gnathostoma* and Gnathostomiasis with special reference to Japan. Progress of Medical Parasitology in Japan. *Exp Parasitol*, 1960, 9, 338-370
30. Miyazaki I. An illustrated book of helminthic zoonoses. *Int Med Found Japan*, 1990, 494p
31. Molin R. Prospectus helminthum, quae in prodromo faunae helminthologicae Venetae continentur. *D K Akad Wissen Wien Math Nat*, 1858, 30, 127-158
32. Owen R. Anatomical description of two species of entozoan from the stomach of a tiger (*Felis tigris* Linn) one of which forms a new genus of Nematoidea *Gnathostoma*. *Proc Zool Soc London*, 1836, 4, 123-126
33. Railliet A, Henry A. Quelques nematode parasites des reptiles. *Bull Soc Pathol Exot*, 1912, 5, 251p
34. Railliet A, Henry A. Sur les oxyrurides. *CR Soc Biol*, 1915, 79, 113p
35. Rautela AS, Malhotra SK. A new rhabdochonid nematode *Comephronema mackiewiczi* n.sp. from a hill stream teleost of a new zoogeographical region. *Riv Parasitol*, 1984, 1, 285-291
36. Rojekittikhun W, Waikagul J, Chaiyasith T. Fish as the natural second intermediate host of *Gnathostoma spinigerum*. *Southeast Asian J Trop Med Pub Hlth*, 2002, 33, 63-69
37. Ruiz JM. Sobre um novo *Gnathostoma* assinalado no Brasil (Nematoda: Gnathostomatidae). *Mem Inst Butan*, 1952, 24, 37-44

38. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol*, 1987, 4, 406-425
39. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual (2nd ed). 1989, 1659p, NY Cold Spring Harbor Laboratory Press
40. Sambrook J, Mac Callum P, Russel D. Molecular cloning: A laboratory manual (2nd ed). 2001, 2100p, NY Cold Spring Harbor Laboratory Press
41. Shrivastav AB, Singh KP, Bhat MA, Mishra A. Occurance of *Gnathostoma spinigerum* in free range tigress. *J Parasit Dis*, 2011, 35, 75-76
42. Sieu TPM, Dung TTK, Nga NTQ, Hien TV, Dalsgaard A, Waikagul J, Murrell KD. Prevalence of *Gnathostoma spinigerum* infection in wild and cultured swamp eels in Vietnam. *J Parasitol*, 2009, 95, 246-248
43. Sohn WM, Lee SH. Identification of larval *Gnathostoma* obtained from imported Chinese loaches. *Korean J Parasitol*, 1996, 34, 161-167
44. Sohn WM, Lee SH. The first discovery of larval *Gnathostoma hispidum* (Nematoda: Gnathostomidae) from a snake host, *Agkistrodon brevicaudus*. *Korean J Parasitol*, 1998, 36, 81-89
45. Stiles C. The determination of generic types and a list of round worm genera, with their original and type species. *US Dept Agri Bur Anim Ind Bull*, 1905, 79, 1-150
46. Stossich M. *Sopra alcuni* nematode della collezione elmintogica del Prof. Dott. Corrado Parona. *Bol Mus Zool Comp Real Univ Genova*, 1902, 116, 1-16
47. Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol*, 2007, 24, 1596-1599
48. Tandon V. Ocular parasitoses: The lesser known and emerging diseases. *Proc 23rd Nat Cong Parasitol,* 2011, Abt Pl06, 4p
49. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgens DG. The Clustam X windows interface: flexible strategies for multiple sequence alignment aided quality analysis tools. *Nucl Acids Res*, 1997, 24, 4876-4882
50. Travassos L. *Gnathostoma americanum* n.sp. *CR Soc Biol*, 1925, 93, 973-974
51. Upadhyay SK. Transmission dynamics and environmental influence on food borne parasitic helminthes of the Gangetic plains and central west coast of India. D.Phil Thesis (awarded) University of Allahabad, India, 2012, 400p
52. Upadhyay SK, Yadav D, Tripathi, A. A redescription and report of *Eustrongylides tubifex* Nitzsch, 1819 (Dioctophymoidea: Dioctophymidae) larvae in deep sea fish *Sillago sihama* off central west coast of India at Goa. *Natl J Life Sci*, 2015, 12, 161-164
53. Upadhyay SK, Jaiswal N, Malhotra A, Malhotra SK. An aspidoderid roundwarm *Pseudaspidodera cordinae* n.sp. from rodents at Allahabad. *Indian J Helminthol*, 2009, 27, 67-72
54. Yadav AK, Tandon V. SEM study of head and cuticular armature of *Gnathostoma doloresi* (Nematoda, Gnathostomatidae). *Acta Parasitol*, 1994, 39, 150-152
55. Yamaguti S. Studies on the helminth fauna of Japan (Pt 35). Mammalian nematodes Vol II. *Jpn J Zool*, 1941, 9, 409-438
56. Yamaguti S. Systema Helminthum Vol III. The Nematodes of Vertebrates. Interscience Publications Inc NY, 1962, 1261p
57. Youn H. Review of zoonotic parasites in medical and veterinary fields in the republic of Korea. *Korean J Parasitol*, 2009, 47, S133-S141
58. Zeder JGH. Erster Nachtrag zur Naturgeschichte der Eigeweidewurmer mit Zufassen und Ammerrkungen herausgegeben. *Leipzig*, 1800, XX+320p